IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

Atty Dkt. LCM-1331-334

C# M#

VON BORSTEL TC/A.U. 1623

Serial No. 09/763,955 Examiner: Patrick T. Lewis

Filed: February 28, 2001 Date: March 2, 2009

Title: COMPOSITIONS AND METHODS FOR TREATMENT OF MITOCHONDRIAL DISEASES

FACSIMILE CERTIFICATE
I hereby certify that this Amendment is being transmitted by facsimile to the Patent and Trademark Office on March 2, 2009, specifically to 571-273-8300.

Signature
Leonard C. Mitchard
Reg. No. 29,009

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

No. of pages transmitted (including this cover sheet): 51 pages

Sir:

SUBMISSION OF CORRECTED APPEAL BRIEF

This is a response/amendment/letter in the above-identified application and includes an attachment which is hereby incorporated by reference and the signature below serves as the signature to the attachment in the absence of any other signature thereon.

☐ **Correspondence Address Indication Form Attached.**

Fees are attached as calculated below:

Total effective claims after amendment 0 previously paid for 20 (at least 20) =	0 minus highest number x $52.00	$0.00 (1202)/$0.00 (2202)	$
Independent claims after amendment 0 previously paid for 3 (at least 3) =	0 minus highest number x $220.00	$0.00 (1201)/$0.00 (2201)	$
If proper multiple dependent claims now added for first time, (ignore improper); add		$390.00 (1203)/$0.00 (2203)	$
Petition is hereby made to extend the current due date so as to cover the filing date of this paper and attachment(s)		One Month Extension $130.00 (1251)/$0.00 (2251) Two Month Extensions $490.00 (1252)/$0.00 (2252) Three Month Extensions $1110.00 (1253/$0.00 (2253) Four Month Extensions $1730.00 (1254/$0.00 (2254) Five Month Extensions $2350.00 (1255/$0.00 (2255)	$
Terminal disclaimer enclosed, add		$140.00 (1814)/ $0.00 (2814)	$

☐ Applicant claims "small entity" status. ☐ Statement filed herewith

Rule 56 Information Disclosure Statement Filing Fee	$180.00 (1806)	$	0.00
Assignment Recording Fee	$40.00 (8021)	$	0.00
Other:		$	0.00
	TOTAL FEE	$	0.00

☐ **CREDIT CARD PAYMENT FORM ATTACHED.**

The Commissioner is hereby authorized to charge any deficiency, or credit any overpayment, in the fee(s) filed, or asserted to be filed, or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Account No. 14-1140.

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By Atty: Leonard C. Mitchard, Reg. No. 29,009

Signature:

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Patent Application of

Reid W. von Borstel, et al — Atty. Ref.: 1331-334

Serial No. 09/763,955 — TC/A.U.: 1623

Filed: February 28, 2001 — Examiner: Lewis, P.T.

For: PYRIMIDINE NUCLEOTIDE PRECURSORS FOR TREATMENT OF SYSTEMIC INFLAMMATION AND INFLAMMATORY HEPATITIS

* *

March 2, 2009

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

CORRECTED APPEAL BRIEF

Sir:

Appellant hereby submits a corrected Appeal Brief and Exhibit 1 in this case to replace that submitted on December 22, 2008. The cover sheet and credit card authorization form filed on December 22, 2008 contained the correct serial number (namely, 09/763,955) but the Appeal Brief itself contained an incorrect serial number. The present corrected Appeal Brief contains the correct serial number (09/763,955).

TABLE OF CONTENTS

(I) REAL PARTY IN INTEREST

The real party in interest is Wellstat Therapeutics Corporation (formerly Pro-Neuron, Inc.), a corporation of the USA of 930 Clopper Road, Gaithersburg, Maryland 20878.

(II) RELATED APPEALS AND INTERFERENCES

The appellant, the undersigned, and the assignee are not aware of any related appeals, interferences, or judicial proceedings (past or present), which will directly affect or be directly affected by or have a bearing on the Board's decision in this appeal, except for the pending appeal in copending application Serial No. 09/930,494, filed August 16, 2001, claims 31-32 and 38-41 of which are the subject of an obviousness-type double patenting rejection with respect to claim 55 of the present application.

(III) STATUS OF CLAIMS

Claims 48-50, 55, 62-64 and 68 are pending and have been rejected. Claims 1-47, 51-54, 56-61 and 65-67 are canceled. No claims have been substantively allowed.

Claims 48-50, 55, 62-64 and 68 are appealed.

(IV) STATUS OF AMENDMENTS

No amendments have been filed since the date of the Final Rejection.

(V) SUMMARY OF CLAIMED SUBJECT MATTER

Claims 48, 49, 50 and 55 are the independent claims in the case. Claim 48 claims a method for treating a congenital mitochondrial disease selected from Mitochondrial Encephalomyopathy, Lactic Acidemia, Leber's Hereditary Optic Neuropathy; Mitochondrial neurogastrointestinal encephalomyopathy; Progressive External Ophthalmoplegia; Leigh's Disease (page 20, beginning at line 7; page 21, lines 1-7) in a mammal by administering to the mammal in need of such treatment an effective amount of a pyrimidine nucleotide precursor (page 5, first complete paragraph) selected from uridine, an acyl derivative of uridine, cytidine and an acyl derivative of cytidine (page 7, last complete paragraph, especially the 4th and 5th lines of that paragraph).

Claim 49 claims a method for treating Alzheimer's Disease (page 28, 2nd and 3rd paragraphs) in a mammal by administering to the mammal in need of such treatment an effective amount of a pyrimidine nucleotide precursor selected from uridine, an acyl derivative of uridine, cytidine and an acyl derivative of cytidine (page 7, last complete paragraph, especially the 4th and 5th lines of that paragraph).

Claim 50 claims a method for treating Huntington's Disease (page 28, 5th paragraph and Example 8) in a mammal by administering to the mammal in need of such treatment an effective amount of a pyrimidine nucleotide precursor selected from uridine, an acyl derivative of uridine, cytidine and an acyl derivative of cytidine (page 7, last complete paragraph, especially the 4th and 5th lines of that paragraph).

Claim 55 claims a method for treating pathophysiological consequences of mitochondrial respiratory chain dysfunction selected from renal tubular acidosis (page

21, line 12; page 30, 2nd complete paragraph; Example 3), dilating cardiomyopathy (page 21, lines 13-14) and lactic acidemia (page 21, lines 1 and 14) in a mammal by administering to the mammal in need of such treatment an effective amount of a pyrimidine nucleotide precursor selected from uridine, an acyl derivative of uridine, cytidine and an acyl derivative of cytidine (page 7, last complete paragraph, especially the 4th and 5th lines of that paragraph).

(VI) GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The ground of rejection to be reviewed on appeal are as follows:

(I) The rejection of claims 48-50, 55, 62-64 and 68 under 35 U.S.C. §112, first paragraph on alleged lack of enablement grounds; and

(II) The obviousness-type double patenting rejection of claim 55 over claims 31-32 and 38-41 of copending Application Serial No. 09/930,494.

(VII) ARGUMENT

I. THE LACK OF ENABLEMENT REJECTION

Claims 48-50, 55, 62-64 and 68 stand rejected under 35 U.S.C. §112, first paragraph, on the ground that the specification, at the time the application was filed, is allegedly not enabling in that it would not have taught one skilled in the art how to make and/or use the full scope of the claimed invention without the exercise of undue experimentation. The rejection is respectfully traversed.

At the outset, the position taken in the Final Action is inconsistent with that previously taken by the Office during prosecution of this case. Thus, in the Action mailed on October 22, 2003, the Office rejected claims 48-59 and 62-68 on lack of enablement grounds, asserting that the specification, "while **enabling**" (emphasis added) for the "treatment" of congenital mitochondrial disease, Alzheimer's Disease, Huntington's Disease, neuromuscular degenerative disease, and pathophysiological consequences of mitochondrial respiratory chain dysfunction, allegedly did not reasonably provide enablement for the "prevention" of congenital mitochondrial disease, Alzheimer's Disease, Huntington's Disease, neuromuscular degenerative disease, and pathophysiological consequences of mitochondrial respiratory chain dysfunction. (Official Action, mailed 10/22/03, page 4).

In response to that 2003 rejection, applicants amended their claims to remove the references to "prevention" of the recited condition. Based on that amendment, the lack of enablement rejection was withdrawn in the immediately following Action mailed September 9, 2004.

The assertion of lack of enablement of the claims directed to the treatment of the recited conditions is not justified by any new reasoning or evidence reflected in the prosecution history of this case. Reversal of the lack of enablement rejection on this ground is respectfully requested.

The Final Action asserts that:

> "At the time of the invention, the treatment of mitochondrial disorders was ineffective. There were no correlations between treatment regimens and therapeutic responses to disorders. Treatment was unpredictable and heterogenous. The examiner directs applicant to PRZYREMBEL *J. Inher. Metab. Dis.* (1987), Vol. 10, pages 129-146 (PRZYREMBEL). PRZYREMBEL teaches, 'Mitochondrial disorders, namely defects of fatty acid oxidation, defects of pyruvate metabolism and defects of the respiratory chain are heterogenous in clinical picture and in response to therapeutic attempts. Defects of fatty acid metabolism are amenable to therapy by dietary means, carnitine substitution and in some cases with vitamins. Defects in pyruvate metabolism do not respond to therapy except in some special cases. Therapeutic attempts include dietary measures, vitamins as coenzyme precursors. Defects in the respiratory chain appear to respond to treatment only in exceptional cases. Evaluation of treatment effects appears to be singularly difficult.' See Abstract." (Final Action, paragraph bridging pages 4 and 5).

The reliance on Przyrembel *J. Inher. Metab. Dis.* (1987), Vol. 10, pages 129-146 (Przyrembel) in support of a lack of enablement rejection is misplaced. At the outset, it is noted that Przyrembel was published in 1987, approximately eight years before the priority date of the present application and at a very early stage in the understanding of mitochondrial diseases and their treatment. Significant progress has been made since 1987 in the understanding of mitochondrial disease and its treatment. In this regard, attention is directed to the Amendment dated October 7, 2005 and the accompanying IDS, which listed (and was accompanied by a copy of), *inter alia*, a reference to DiMauro

et al., "Mitochondrial Encephalomyopathies: Where Next?" (1999) *Revista De Neurologia*, 28(2):164-168 (DiMauro) (Exhibit 1 hereto). In that reference, it is stated at page 2:

> "Although mtDNA was discovered 36 years ago (Nass and Nass 1963) and human mtDNA had been fully sequenced by 1981 (Anderson, et al. 1981), clinicians paid no attention to this genetic relic until **1988**, when mutations in mtDNA were first associated with human disease; (Holt, et al. 1988, Wallace, et al, 1988). In the intervening years, however, a cadre of scientists had worked hard at clarifying the organization of the mitochondrial genome and the peculiar rules governing its replication, transcription, and translation (Schon 1997).....As the title of this chapter implies, we will not even attempt to review the **enormous progress achieved** in the 38 years since Luft and coworkers introduced the concept of mitochondrial disease, nor even **in the 12 years since the description of pathogenic mtDNA mutations.**" (Emphasis added)

In light of the above, it is clear that one of ordinary skill would **not** have viewed Przyrembel (1987) as indicative of the state of the art against which the presently claimed invention should be gauged. DiMauro notes the "enormous progress" made since Luft's initial work, and one of ordinary would have been aware of this as of the filing of the present application. Although DiMauro was published about one year after the priority date of the subject application, DiMauro is evidence of developments in the art during the period between publication of Przyrembel (1987) and the priority date of the subject application. Moreover, Examples 1, 3, 7, 8 and 9 of the present application all deal with respiratory chain defects and further support enablement of the invention as claimed. Thus, any assertion that it is "only in exceptional cases" (Final Action, page 5, line 7) that defects in the respiratory chain appear to respond to treatment is no longer true in light of the instant invention. The person of ordinary skill in the art, upon reading the

specification of the subject application, would consider any such statement in Przyrembel to have been superseded. For these further reasons, it is believed that the lack of enablement rejection should be reversed.

In addition, in an effort to progress prosecution, the claims have been amended during prosecution, without prejudice, to specify that the pyrimidine nucleotide precursor is selected from uridine, an acyl derivative of uridine, cytidine and an acyl derivative of cytidine. Support appears in the originally filed application at pages 7 and 8.

Reversal of the lack of enablement rejection is clearly in order, and is requested.

II. OBVIOUSNESS-TYPE DOUBLE PATENTING

Claim 55 stands provisionally rejected on obviousness-type double patenting grounds as allegedly unpatentable over claims 31, 32 and 38-41 of copending Application Serial No. 09/930,494. Applicants will consider filing a Terminal Disclaimer when otherwise allowable subject matter is indicated.

Favorable action is awaited.

Respectfully submitted,

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(VIII) CLAIMS APPENDIX

1-47. (cancelled)

48. A method for treating a congenital mitochondrial disease selected from the group consisting of Mitochondrial Encephalomyopathy, Lactic Acidemia, Lerber's Hereditary Optic Neuropathy; Mitochondrial neurogastrointestinal encephalomyopathy; Progressive External Ophthalmoplegia; Leigh's Disease; in a mammal comprising administering to said mammal in need of such treatment an effective amount of a pyrimidine nucleotide precursor selected from the group consisting of uridine, an acyl derivative of uridine, cytidine and an acyl derivative of cytidine.

49. A method for treating Alzheimer's Disease in a mammal comprising administering to said mammal in need of such treatment an effective amount of a pyrimidine nucleotide precursor selected from the group consisting of uridine, an acyl derivative of uridine, cytidine and an acyl derivative of cytidine.

50. A method for treating Huntington's Disease in a mammal comprising administering to said mammal in need of such treatment an effective amount of a pyrimidine nucleotide precursor selected from the group consisting of uridine, an acyl derivative of uridine, cytidine and an acyl derivative of cytidine.

55. A method for treating pathophysiological consequences of mitochondrial respiratory chain dysfunction selected from the group consisting of renal tubular acidosis, dilating cardiomyopathy and lactic acidemia in a mammal comprising administering to said mammal in need of such treatment an effective amount of a pyrimidine nucleotide precursor selected from the group consisting of uridine, an acyl derivative of uridine, cytidine and an acyl derivative of cytidine.

62. A method as in claim 48 wherein said acyl derivative of uridine is 2',3',5'-tri-O-acetyluridine.

63. A method as in claim 49 wherein said acyl derivative of uridine is 2',3',5'-tri-O-acetyluridine.

64. A method as in claim 50 wherein said acyl derivative of uridine is 2',3',5'-tri-O-acetyluridine.

68. A method as in claim 55 wherein said acyl derivative of uridine is 2',3',5'-tri-O-acetyluridine.

(IX) EVIDENCE APPENDIX

Exhibit 1: DiMauro *et al.*, "Mitochondrial Encephalomyopathies: Where Next?" (1999) *Revista De Neurologia*, 28(2):164-168 (acknowledged in the initialed IDS attached to the Official Action dated November 23, 2005).

(X) RELATED PROCEEDINGS APPENDIX

None.

1999 REVISTA DE NEUROLOGIA 28(2):164-168

MITOCHONDRIAL ENCEPHALOMYOPATHIES: WHERE NEXT?

Salvatore DiMauro, Kurenai Tanji, Eduardo Bonilla,
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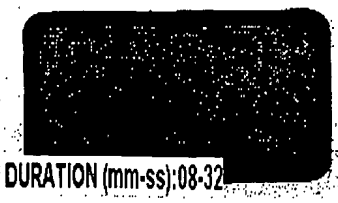
Introduction

There are papers in the history of medicine that will never become obsolete because they combine original thinking with the best technology available at the time to open up new vistas. One such paper is the report in 1962 by Luft and coworkers of a young Swedish woman with severe hypermetabolism, mild weakness, and normal thyroid function (Luft, et al. 1962). The genial intuition of Rolf Luft, an endocrinologist, that the problem was to be sought in skeletal muscle rather than in the thyroid gland, combined with the expertise in mitochondrial bioenergetics of the late Lars Ernster and the morphological acumen of Bjorn Afzelius, led the Karolinska group to discover the first mitochondrial disease and the first example of "organellar medicine". The fact that Luft disease is one of the rarest human metabolic disorders is a historical curiosity that does not diminish the importance, indeed the beauty, of that observation.

What Luft may not have suspected at the time was that he was opening a seemingly bottomless Pandora's box of human diseases. By 1994, it was clear that mitochondrial dysfunction could affect every tissue in the body, thus amply justifying the term "mitochondrial medicine" that Luft introduced in the title of a review article (Luft 1994). At this meeting, he gave us a fascinating account of the discovery of Luft disease and an update on "mitochondrial medicine".



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Introduction

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What Luft may not have suspected at the time was that he was opening a seemingly bottomless Pandora's box of human diseases. By 1994, it was clear that mitochondrial dysfunction could affect every tissue in the body, thus amply justifying the term "mitochondrial medicine" that Luft introduced in the title of a review article (Luft 1994). At this meeting, he gave us a fascinating account of the discovery of Luft disease and an update on "mitochondrial medicine".

Of course, one of the unique characteristics of mitochondrial diseases is that mitochondria are relics of independent bacteria-like intruders (welcome intruders, as it turned out) that took permanent residence in our cells over a billion years ago. As such, mitochondria possess their own DNA (mtDNA) and are under dual genetic control. If we focus our attention on the "business end" of mitochondrial energy metabolism, the respiratory chain, only 13 of its approximately 90 proteins are encoded by mtDNA and are synthesized within the organelle, whereas all others are encoded by nuclear DNA (nDNA). After being synthesized in the cytosol, the nDNA-encoded subunits are imported into the organelle, where they are assembled, together with their mtDNAencoded counterparts, into the respective holoenzymes in the mitochondrial inner membrane.

Although mtDNA was discovered 36 years ago (Nass and Nass 1963) and human mtDNA had been fully sequenced by 1981 (Anderson, et al. 1981), clinicians paid no attention to this genetic relic until 1988, when mutations in mtDNA were first associated with human disease; (Holt, et al. 1988, Wallace, et al. 1988). In the intervening years, however, a cadre of scientists had worked hard at clarifying the organization of the mitochondrial genome and the peculiar rules governing its replication, transcription, and translation (Schon 1997). One of the pioneers in this field was Giuseppe Attardi at the California Institute of Technology. His participation in this meeting illustrates another interesting aspect of mitochondrial pathology: the close collaboration between basic and clinical scientists. The lack of spontaneous or engineered animal models for mtDNA related diseases has made patients and patient-derived cells precious commodities where concepts derived from *in vitro* studies could be verified *in vivo* or, at least, in tissue culture, such as the cybrid system introduced by King and Attardi (King and Attardi 1989).

As the title of this chapter implies, we will not even attempt to review the enormous progress achieved in the 38 years since Luft and coworkers introduced the concept of mitochondrial disease, nor even in the 12 years since the description of pathogenic mtDNA mutations. In trying to gaze into the future, we will discuss four

subjects of special interest to our group, where recent information has made us revise old "dogmas" or has opened up new areas of investigation. In keeping with the concept that the term "mitochondrial encephalomyopathy" refers to defects of oxidative phosphorylation, and that the respiratory chain is under the control of two genomes, we will consider two subjects related to mtDNA and two related to nDNA.

I. Have we neglected the mtDNA protein-coding genes?

During the past 12 years, experience with a rapidly increasing population of patients harboring mtDNA mutations has led clinical investigators to formulate a number of generalizations that are too often interpreted as "dogmas". Following is a short list of such dogmas.

1. Point mutations in mtDNA are transmitted by maternal inheritance and negative family history is evidence against such etiology.

2. mtDNA point mutations are associated with multisystem disorders and are rarely strictly tissue-specific.

3. Ragged-red fibers (RRF), the histochemical hallmark of massive mitochondrial proliferation in muscle, are typically seen in patients with mtDNA mutations that impair overall mitochondrial protein synthesis, such as mutations in tRNA or rRNA genes, single or multiple deletions, or mtDNA depletion. Conversely, RRF are absent in muscle biopsies from patients with mutations in mtDNA protein-coding genes, such as the NARP/MILS mutations in the ATPase 6 gene, or the various mutations in genes encoding complex 1 subunits (ND genes) associated with Leber's hereditary optic neuropathy (LHON).

4. In patients with mtDNA point mutations, RRF do not show cytochrome c oxidase (COX) activity (i.e. are histochemically COX-negative), except in patients with typical MELAS syndrome, where they are COX-positive. All of these dogmas have been shattered, or at least cracked, by recent experience.

1. Point mutations in protein-coding genes often arise de novo and cause sporadic disorders. Examples abound and are illustrated in Tables 1 and 2.

Among *complex* I gene defects, only two have been found in sporadic cases thus far: a nonsense mutation in the ND4 gene (Andreu, et al. 1999c), and an intragenic inversion in the NDI gene altering three highly conserved amino acids (Musumeci et al, in preparation). Both patients suffered from exercise intolerance and one had minimal proximal limb weakness.

Mutations in cytochrome *b*, the only mtDNA-encoded subunit of *complex III*, offer the best example of this apparently anomalous situation (jable 1). Nine sporadic patients, all with exercise intolerance and two with recurrent myoglobinuria, had pathogenic mutations in the cytochrome *b* gene (Dumoulin et al. 1996; Andreu et al. 1998; Kennaway et al. 1998; Andreu et al. 1999a; Andreu, et al. 1999b). All mutations were different, though all were G-to-A transitions. However, not all pathogenic mutations in the cytochrome *b* gene are spontaneous events: a maternally inherited multisystem disorder with features of MELAS and parkinsonism was associated with a microdeletion (De Coo et al. 1999).

When it comes to defects of *complex* IV (cytochrome c oxidase, COX), the variety of clinical presentations is greater, but, again, maternal inheritance is extremely rare, having been seen in only one of nine reported patients (Table 2).

2. Point mutations in protein-coding genes are often tissue-specific.

The one tissue that is most often affected is skeletal muscle, suggesting that these are somatic mutations, that is, spontaneous events that arose in myoblasts or in myoblast precursors after germ-layer differentiation. Pure myopathy, dominated by exercise intolerance with or without myoglobinuria and sometimes associated with mild limb weakness, characterized two patients with *complex I deficiency*, all nine patients with *complex III deficiency* (jable 1), and two patients with COX *deficieney*

(jable 2).

Exercise intolerance is a common complaint, which, if the patient has no objective weakness, increased serum creatine kinase (CK) levels, or abnormal electromyography (EMG), is often dismissed as "psychogenic" or mislabeled as "chronic fatigue syndrome"or "fibromyalgia rheumatica". Many patients with mutations in mtDNA protein-coding genes fall into this group, and the lack of maternal inheritance further distracts the physician from thinking about mitochondrial dysfunction. It is important, when faced by these puzzling patients, to consider the possibility of a mitochondrial disease and to obtain, at the very least, a resting lactate value. Increased lactate was the only abnormality that led an astute clinician to biopsy a young man with life-long exercise intolerance. The muscle biopsy showed RRF and markedly decreased complex I activity, and molecular genetic analysis of muscle mtDNA revealed a- nonsense mutation in the ND4 gene(Andreu et al. 1999c).

Myoglobinuria, and especially recurrent myoglobinuria, is commonly associated with blocks in the utilization of the two major sources of energy for muscle contraction, glycogen or fatty acids (DiMauro and Haller 1999). Strangely, blocks in oxidative phosphorylation, the final common pathway for energy production, were not considered until recently in the differential diagnosis of myoglobinuria. However, two patients with *complex III deficiency* did have each one episode of myoglobinuria (Andreu et al. 1999a, Andreu et al. 1999b) whereas both patients with myopathy and COX *deficiency* had multiple episodes of myoglobinuria related to unusually intense or repeated exercise ; Karadimas et al. 1999). The difference in the frequency and intensity of myoglobinuria attacks between patients with defects in complex HI and IV suggests that COX deficiency causes a more severe "energy crisis". For the sake of completion, we should mention one other respiratory chain defect often associated with myoglobinuria, coenzyme Q10 (CoQ10) deficiency (Ogasahara et al. 1989, Servidei et al. 1996, Sobreira et al. 1997). However, CoQ10 is not encoded by mtDNA and primary defects of CoQ10 are presumably due to mutations in

nuclear genes encoding one or more biosynthetic steps.

In two patients with sideroblastic anemia and COX deficiency, distinct but closely located mutations in the COX 1 gene were found in bone marrow, whole blood, isolated platelets, and granulocytes, but not in T or B lymphocytes, buccal mucosa, or skin fibroblasts (Gattermann et al. 1997). This pattern suggested that the mutations occurred *de novo* in bone marrow stem cells with myeloid determination. The fact that tissues other than muscle can be selectively affected suggests that we should keep an open mind about the possibility that somatic mutations of mtDNA protein-coding genes may be involved in other tissue-specific disorders, such as cardiopathies or encephalopathies.

3. Ragged-red fibers are commonly associated with mutations in protein-coding genes. This is especially evident in patients with complex I and complex In deficiencies. Both patients with *complex I deficiency* (Andreu et al. 1999c), and all but one patient with *complex III deficiency* (Table 2) had RRF, which stained intensely for COX (COX positive RRF). The only exception was a 38-year-old woman. with exercise intolerance, proximal weakness, and a missense mutation in the cytochorne *b* gene (Andreu, et al. 1998). In patients with *complex IV deficiency,* RRF are generally less abundant and can even be absent. Not surprisingly, those RRF that there are, are COX-negative, and there is an abundance of COX-negative non-ragged-red fibers. These findings cast some doubts on the hypothesis that impairment of mitochondrial protein synthesis is the trigger for mitochondrial proliferation.

2. Why is MELAS different from KSS?

This "tongue-in-cheek" question actually reflects our woeful ignorance about the pathogenesis of mtDNA-related diseases, and especially those due to mtDNA rearrangements and to mutations in tRNA genes. There is ample evidence from studies of patients' tissues and of cybrid cell lines that both types of mutations impair mitochondrial protein synthesis and affect one and the same pathway, the

respiratory chain. Why, then, are there so many different clinical presentations, some of which are stereotypical enough to be easily recognizable at the bedside and amenable to acronymic labeling, such as MELAS, MERRF, or KSS? Some answers are provided by the peculiar rules of mitochondrial genetics. Thus, varying degrees of heteroplasmy in different tissues coupled with different thresholds of vulnerability to oxidative iMpairment can explain much of the inter-organ variability in these disorders.

However, questions become harder when it comes to the brain. Why are certain brain functions typically affected in MELAS while others are predominantly affected in MERRF and still others in KSS? For example, the stroke-like lesions of MELAS are only rarely seen in MERRF or KSS; conversely, myoclonus is fairly typical of MERRF; and seizures are virtually obligatory in both MELAS and MERRF but are very rare in KSS. Given the cellular heterogeneity and organizational complexity of the brain, we have posited that uneven spatial degrees of heteroplasmy for different mutations might explain some of the differential neurological signs. With this working hypothesis, we have started to draw "morbidity maps" of the brain in the different mitochondrial encephalomyopathies based on comparative immunohistochemistry, using antibodies against mtDNA-encoded respiratory chain proteins (e.g. COX H or NDI) and nDNAencoded proteins (e.g. COX IV or the non-heme iron-sulfur protein of complex 111).

This approach is yielding some interesting results. When we studied the dentate gyrus of the hippocampus, a highly epileptogenic area (Dichter and Buchhalter 1997), from. two patients, one with MELAS the other with KSS, we found that immunoreactivity for the FeS-protein was similar in both whereas immunoreactivity for COX II was markedly reduced in the patient with MELAS but normal in the patient with KSS (Figure 1). Although indirect, these data suggest that the A3243G MELAS mutation was abundant enough in the dentate gyrus to curtail mitochondrial protein synthesis, while the mtDNA deletion in the KSS patient must have been below the threshold needed to affect COX H biosynthesis. An even more indirect,

but not unreasonable, conclusion is that the relative sparing of the hippocampus in KSS may contribute to explain the rare occurrence of seizures in this condition.

We next asked ourselves if a similar spatially selective nútochondrial dysfunction could explain cerebellar ataxia, another common symptom of mitochondrial encephalomyopathies, and especially of MERRF and KSS.

In normal brains, immunohistochemistry of the cerebellar system with anti-COX II and anti-FeS antibodies showed finely punctate reaction in the cortex, the dentate nucleus, and the olivary nucleus. In the cerebellar cortex, the immunostain was evenly distributed throughout the molecular layer, showed a dense granular pattern in the glomeruli of the internal granular layer, and decorated clearly both perikaria and apical dendrites of Purkinje cells. The shapes of the dentate and olivary nuclei were neatly outlined by the immunoreaction of the neuropil, and large multipolar neurons reacted strongly with both antibodies (Figures 2, 3, and 4 .

In both MERRF and KSS, the immunoreactivity of the cerebellar cortex was similar to that of control brains with both antibodies (Figure 2). In KSS, however, immunostain with COX II antibodies was markedly decreased in the dentate nucleus, in contrast to the normal reaction with FeS antibodies ffigure 3). In MERRF, this selective immunostaining defect for the mtDNA-encoded protein included not only the dentate but also the olivary nucleus (Figures 3 and 4). Thus, we have strong immunochernical evidence of mitochondrial dysfunction in cerebellar structures that may play an important role in the pathogenesis of ataxia. The dentate nucleus is an important relay station in the cerebello-thalamo-cortical pathway, which receives axons from the Purkinje cells of the cerebellar hemispheres. The inferior olivary nucleus appears to be the pacemaker station of the cerebellar system, where climbing fibers originate on their way to innervate Purkinje cells. Our evidence that the A8344G MERRF mutation accumulates in both dentate and olivary nuclei while deleted mtDNAs are especially abundant in the dentate nucleus suggests that there may be a major disconnection of the cortico-nuclear cerebellar

system at least at the level of the dentate nucleus. This disconnection may play a major role in the pathogenesis of ataxia in patients with KSS and MERRF (Tanji et al. 1999).

Although some interesting immunopathological brain "maps" are emerging, this still begs the question of which factors are responsible for the putative selective spatial abundance of distinct mutations in different areas of the brain.

3. Mitochondrial diseases: back to mendelian genetics

For the past decade and until recently, mtDNA-related disorders seemed to be "the only game in town": novel mtDNA mutations were described seemingly every week, and the clinical expression of mtDNA mutations was fascinating in its variety. As discussed above, much remains to be done before we fully understand the pathogenetic mechanisms of mtDNA mutations. However, mtDNA is small and we may be scraping the bottom of the barrel searching for new mutations. The time has come to direct our attention to the more difficult task of identifying nuclear DNA mutations responsible for respiratory chain defects. This is particularly important because two of the four most common causes of Leigh syndrome, a devastating neurodegenerative disease of infancy or childhood, aredue to specific respiratory chain defects: complex I deficiency (Rahman et al. 1996; Kirby et al. 1999), and COX deficiency (Willems, et al 1977; DiMauro et al. 1987; Van Coster et al. 1991). Both conditions are inherited as autosomal recessive traits. The other two common causes of Leigh syndrome are pyruvate dehydrogenase complex (PDHC) deficiency and the T8993G mutation in the mtDNA ATPase 6 gene. PDHC deficiency is usually inherited as an X-linked dominant trait while the T8993G mutation is the most common cause of maternally inherited Leigh syndrome (MILS).

This shift in interests towards the nuclear genome is yielding exciting results, and it has generated fancifully titled editorials, such as "Nuclear power and mitochondrial

disease" (DiMauro and Schon 1998), "Getting to the nucleus of mitochondrial disorders..." (Dahl 1998), "Mitochondrial encephalomyopathies: back to Mendelian genetics" (DiMauro 1999). Table 3 summarizes the considerable amount of information that has already been gathered on nuclear mutations and respiratory chain defects.

We have had a long-standing interest in COX deficiencies and COX-deficient Leigh syndrome (LS). There are ten nDNA-encoded subunits of COX (IV, Va, Vb, VIa, VIb, Vic, V1Ia, V11b, VIIc, and VHI, according to the nomenclature of Kadenbach et al]), and we reasoned that COX-deficient LS could be due to mutations in one or more of the nuclear-encoded genes specifying non-tissue-specific COX subunits. We sequenced all nDNA-encoded genes in four LS patients and found no abnormalities (DiMauro et al. 1994). Similar negative data were later reported in a single patient (Adams et al. 1997). These findings were in agreement with older biochemical data in three patients of ours,showing decreased v_{max} but normal K. for the residual COX, and normal Northern blot analyses for all 13 COX subunits (Lombes et al. 199 1). Taken together, our biochemical and molecular data led us to conclude that " ... COX deficiency in LS may not be due to a defect in the enzyme *per se*, but rather to the mutation of a nuclear regulatory gene controlling the assembly or stability of the enzyme."(DiMauro, et al. 1994). We went on to say that " ... in yeast, as many as 14 different nuclear genes are needed for proper COX assembly; analogous genes probably exist in humans and they are attractive candidates for the genetic lesion or lesions causing LS". Although we were accurate prophets, others before us have identified the first mutations in one such COX-assembly gene (Zhu et al. 1998; Tiranti et al. 1998).

Thanks largely to the work of Tzagoloff at Columbia University, more than 30 different complementation groups for COX assembly have been identified in yeast (Tzagoloff and Dieckmann 1990). Although many of these genes probably do not have mammalian homologues, a certain number do (Petruzzella et al. 1998) and one or more of them could be the culprits in COX-deficient LS. Because sequencing

each candidate gene is a tedious and uncertain task, and because LS families are too small for traditional linkage analysis, Shoubridge's group resorted to functional complementation of the enzyme defect in cells from LS patients, using microcell-mediated chromosome transfer. They showed that transfer of chromosome 9 into COX-defective cells restored COX activity, and, using deletion mapping, localized the genetic defect to a 4.5 cM region of chromosome 9q34, containing the candidate gene *SURF-1*. Sequence of *SURF-1* in patients revealed five different pathogenic mutations, all of which predicted a truncated protein (Zhu et al. 1998). Soon thereafter, and using a similar approach, the group of Zeviani identified five more distinct mutations in the same gene (Tiranti et al. 1998).

The product of *SURF-1* is indeed a mitochondrial protein, which appears to act at the third stage in the four-step process of COX assembly (Zeviani et al. 2000). Northern and Western blot analyses in tissues from patients with *SURF-1* mutations failed to show any COX transcript or protein, suggesting that these loss-of-function mutations are associated with mRNA instability, rapid protein degradation, or both (Zeviani et al. 2000).

Although the discovery of *SURF1* mutations in COX-deficient LS was a major breakthrough, the story clearly does not end there, because these mutations were not found in all patients. Besides, we had known all along that COX-deficient LS was clinically and biochemically heterogeneous: for example, we had seen a few patients with severe congenital cardiomyopathy in whom COX activity was only partially decreased in liver and fibroblasts (DiMauro, et al. 1987). We decided, therefore, to study COXassembly genes other than *SURF1* in these unusual patients, and we found pathogenic mutations in the SC02 gene in three infants with fatal infantile cardiomyopathy and encephalopathy, but without the typical neuropathological features of LS (Papadopoulou et al. 1999). The SC02 gene encodes a copper-binding protein that must play a crucial role in the assembly of COX, which contains two copper atoms. This essential function coupled with Northern blot evidence that the SC02 protein is expressed predominantly in heart and muscle explains both the

clinical phenotype and the extremely low levels of COX activity found in heart and muscle.

We have screened our series of 41 patients with undiagnosed encephalomyopathies and COX deficiencies for *SURF-1* and SC02 mutations (Sue et al. 2000). We found six patients with *SURF-1* mutations and three with SC02 mutations, and a comparison of the two groups revealed some interesting distinguishing features. First, at the clinical level, all patients with SURF-1 mutations had typical clinical and pathological features of LS, whereas all three patients with SC02 mutations died in early infancy of fulminant cardiopathy. Second, histochemistry of muscle biopsies showed much more severe COX deficiency in patients with SC02 mutations, a finding confirmed by biochemical assays. Third, immunohistochemistry indicated a similar decrease of nDNA-encoded and mtDNA-encoded COX subunits in *SURF-1* patients, but a more severe decrease of mtDNA-encoded proteins in SC02 patients.

It is noteworthy that only 14% of our patients with COX-deficient LS had *SURF 1* mutations, in sharp contrast to 75% in Tiranti's series (Tiranti et al. 1999). The two main reasons for this discrepancy may be that Tiranti's patients belonged to a single complementation group (Munaro et al. 1997) and to a relative uniform ethnic group.

Irrespec tive of their exact frequency, knowledge of two genetic defects associatedwith COX-deficient LS syndrome will now make prenatal diagnosis possible for parents who have often lost one or more children to the disease. On the other hand, the molecular defect remains unknown in a substantial number of COX-deficient LS patients. Screening additional COX-assembly genes for pathogenic mutations is the obvious next step and one that will undoubtedly bear fruit.

4. Defects of intergenomic signaling: the lesson of MNGIE

A special group of mendelian mitochondrial diseases reflects the gradual loss of autonomy of the mitochondrial genome, which now depends heavily on factors encoded by nuclear genes for some of its essential functions, including transcription,

translation, and replication. Disorders of intergenomic signaling are due to mutations in nuclear genes that, directly or indirectly, control mtDNA number, function, or integrity.

The first example of such faulty "dialogue" between the two genomes was offered by patients with autosomal dominant progressive external ophthalmoplegia (PEO) and multiple mtDNA deletions in muscle (instead of the single type of mtDNA rearrangement that characterizes each patient with KSS or sporadic. PEO) (Zeviani et al. 1989). Although linkage analysis has provided both chromosomal localization and evidence of genetic heterogeneity in some families with autosomal dominant PEO (Suomalainen et al. 1995; Kaukonen et al. 1996), no genes responsible for this syndrome have yet been identified.

The second major disorder of intergenomic communication, resulting in tissue specific paucity of mtDNA copies ("mtDNA depletion"), was described in infants with severe congenital myopathy or hepatopathy (Moraes et al. 1991). There are milder myopathic forms of mtDNA depletion (Tritschler et al. 1992) and the clinical spectrum may involve both central and peripheral nervous systems (Vu et al. 1998). The genetic defect (or defects), which presumably impair mtDNA replication, remain elusive. Decreased levels of mitochondrial transcription factor A (mtTFA) in tissues from patients (Larsson et al. 1994; Poulton et al. 1994) probably are a consequence rather than the cause of mtDNA depletion. A partial defect of polymerase -y was reported in a singlepatient with Alpers syndrome and mtDNA depletion (Naviaux et al. 1999), but polymerase y deficiency has not been found in patients with typical mtDNA depletion.

The first defect of intergenomic signaling whose molecular defect has recently been identified is an autosomal recessive form of PEO known by the cacophonic acronym MNGIE, for mitochondrial neurogastrointestinal encephalomyopathy (Hirano et al. 1994). In contrast to the autosomal dominant forms of PEO, which are largely confined to muscle, autosomal recessive PEO syndromes with multiple mtDNA

deletions tend to be multisystemic (Carrozzo, et al. 1998). This is well illustrated by MNGIE, a syndrome dominated by gastrointestinal problems (chronic diarrhea, intestinal pseudoobstruction) leading to cachexia and early death. Additional symptoms and signs includeptosis and ophthalmoplegia, peripheral neuropathy, and leukoencephalopathy. Muscle biopsy shows COX-negative RRF, biochemical evidence of COX deficiency, and molecular evidence of mtDNA multiple deletions, sometimes associated with mtDNA depletion (Bardosi et al. 1987; Hirano et al. 1994; Papadimitriou et al. 1998). First, linkage analysis of four ethnically distinct families with typical MNGIE localized the gene to chromosome 22q13.32-qter (Hirano et al. 1998). Sequencing of candidate genes in this region led to the discovery of homozygous or compound heterozygous mutations in the gene specifying thymidine phosphorylase (TP) in 12 MNGIE patients (Nishino et al. 1999). TP is widely expressed in human tissues, including some that are selectively involved in MNGIE, such as the gastrointestinal system, brain, and peripheral nerves. Paradoxically, however, TP is not expressed in skeletal muscle, which is also affected in MNGIE clinically and which harbors multiple mtDNA deletions. This suggests that the mtDNA abnormalities in MNGIE may be a secondary phenomenon, possibly related to damage by abnormal extracellular thymidine pools. One practical advantage of knowing the gene defect is the availability of a simple biochemical diagnostic assay, based on TP activity in leukocytes, which is virtually undetectable in patients (Nishino et al. 1999). Another practical implication is the possibility that correcting the imbalande of the nueleotide pools might have therapeutic value in this otherwise intractable and devastating disorder.

5. Where next?

The four subjects discussed above illustrate, we hope, both the exciting progress of research in the area of mitochondrial diseases and the challenging problems that still face us. So, where do we go next? Here are some of the many avenues wide open to the curious clinical investigator:

I. Mitochondrial genome: are we scraping the bottom of the barrel? Most emphatically, no! We have documented how mutations in protein-coding genes have been relatively neglected and we predict that more attention to these genes will yield a rich harvest.

The pathogenic mechanisms of mtDNA mutations remain largely elusive, especially the apparent "symptom specificity" of some mutations (Schon et al. 1997).

We do not understand why RRF are much more common in patients with mtDNA than in patients with nDNA mutations. For that matter, we still do not know what triggers mitochondrial proliferation and the formation of RRF

2. Nuclear genome: the search for nuclear gene mutations causing defects in respiratory chain complexes has just started with a bang and promises to be very exciting. The experience with COX deficiency warns us about the possibility of "murder by proxy", that is, gene defects only indirectly related to the respiratory complex in question. Conversely, it is surprising that mutations in nuclear genes encoding COX subunits have not yet been found, and it is a safe bet that sooner or later some such genetic error will be associated with one or more COX-deficiency syndrome.

Nuclear genes not only codify most respiratory chain subunits and assembly factors, but also numerous factors needed for mtDNA replication and integrity. The recent discovery of the molecular basis of MNGIE bodes well for similar breakthroughs in other defects of intergenomic signaling.

Nuclear genes also encode the whole complex machinery needed for mitochondrial protein importation. Although a handful of defects in 1eader peptides", needed for the recognition of mitochondrial proteins by the organelle, have been reported (Fenton 1995), defects of the general importation system are still largely "terra incognita". However, defects of the chaperonin heat shock protein 60 (hsp60) have been described in two children with mitochondrial encephalomyopathy (Agsteribbe

et al. 1993, Briones et al. 1997), and a mutation in DDP, a protein similar to the yeast TIM (translocase of the inner membrane) family of translocases, has been identified in patients with the deafness and dystonia syndrome (Koehler et al. 1999). The stage is set for still another subgroup of mitochondrial diseases to be defined at the clinical and molecular level.

Transport of metabolites across the inner mitochondrial membrane requires a whole battery of translocases encoded by the nuclear genome. These have been largely neglected by clinical researchers, with the exception of the carnitine-acylcamitine carrier (CAC). CAC is part of the "carnitine cycle", feeding long-chain fatty-acyl-CoAs into the mitochondrial P-oxidation pathway, and several patients with biochemically identified CAC deficiency had been reported. Only recently, however, has the gene for CAC been cloned (Huizing et al. 1997) and the first molecular defect identified in a patient with CAC deficiency (Huizing et al. 1998). In addition, reduced amounts of the voltage dependent anion channel (VDAC) of the outer mitochondrial membrane were documented by Western blot in a patient with psychomotor retardation, although the molecular defect was not elucidated (Huizing et al. 1996). Putative genetic defects of the inner membrane adenine nucleotide translocators (ANTs) would be of more direct relevance to mitochondrial disorders due to respiratory chain dysfunction. This concept has been borne out by a knockout mouse deficient in the heart/muscle isoform of ANT (ANT1), an excellent model for human mitochondrial myopathy and cardiopathy (Graham et al. 1997).

3. Defects of the respiratory chain physical milieu. There is ample evidence that the functioning of respiratory chain complexes depends on the integrity of the phospholipid milieu in which they are embedded, especially on a normal amount and structure of cardiolipin (Schlame et al. 1999). Thus, it is at least theoretically possible that patients with multiple respiratory chain defects may have genetic or acquired abnormalities of the inner membrane phospholipids rather than mtDNA mutations. One example of this scenario may be Barth syndrome, an X-linked recessive disorder characterized by cardiopathy, myopathy, leukopenia, and

multiple respiratory chain defects in muscle (Barth et al. 1999). Interestingly, the gene responsible for Barth syndrome,TAZ, encodes a protein called tafazzin, which has sequence homology to a superfamily of acyltransferases active in phospholipid biosynthesis (Barth et al. 1999). Although the alteration in the phospholipid composition of the inner mitochondrial membrane remains to be documented in Barth syndrome, this concept has exciting implications for this and other mitochondrial eneephalomyopathies.

4. Therapy. To the frustration of fan-ilies and doctors, therapy of respiratory chain disorders remains woefully inadequate and usually limited to the administration of various vitamins and cofactors. Gene therapy is still a distant possibility for nuclear defects and is daunting for mtDNA gene defects. However, because of heteroplasmy and the threshold effect, if we could cause even a small shift in the relative percentages of mutant and wild-type mtDNAs, we might affect the clinical phenotype dramatically. Various strategies are being cosidered, including the use of peptide nucleic acids (PNAs) to inhibit the replication of complementary mutant mtDNAs (Taylor et al. 1997), or pharmacologic approaches directed to the same end (Manfredi et al. 1999). The observation that myoblasts, the progenitor muscle cells, often contain lesser amounts ofpathogenic mtDNA mutations than mature muscle fibers (Clark et al. 1997; Shoubridge et al. 1997) has suggested the use of exercise as a mean of inducing limited muscle necrosis, which would be followed by regeneration of muscle fibers harboring lower mutational loads (Taivassalo et al. 1999). This approach could be particularly useful in patients with protein-coding mtDNA gene mutations limited to the musculature (see above - section 1). Thus, there are some promising experimental approaches, but therapy in general remains a major challenge for future researchers.

5. Genetic counseling. This is another frustrating aspect of the mitochondrial diseases. While recent discoveries of mutations in nuclear genes responsible for Leigh syndrome or mitochondrial encephalocardiomyopathy are offering couples the option of prenatal diagnosis, this remains problematic for disorders due to mtDNA

mutations. The lack of a clear correlation between mutational loads in amniocytes or chorionic villi and other fetal tissues for the most common pathogenic tRNA mutations, such as those associated with MELAS and MERRF, makes prenatal diagnosis in these conditions impossible. Theoretically, the nucleus could be removed from the ovum of an asymptomatic or oligosymptomatic woman harboring the MELAS mutation and transplanted into a normal enucleated oocyte. Fertilization and implantation of the manipulated ovum would result in a child with the mother's nuclear genome and the donor's mitochondrial genome (Rubenstein et al. 1995). Obviously, such germ-line therapy raises a number of experimental and ethical issues. In contrast to tRNA gene mutations, the NARP/MILS mutation (T8993G) in the ATPase 6 gene of mtDNA appears to be uniformly distributed in different tissues (White et al. 1999b), thus making prenatal diagnosis feasible (White et al. 1999a).

Clearly, mitochondrial diseases, introduced forty years ago by Luft and coworkers, remain a fertile area of investigation for clinical investigators and will keep them busy well into the new century, if not the new millennium.

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Patient	1	2	3	4	5	6	7	8	9
Sex/age	M/43	F/52	F/38	M/32	M/51	M/28	F/23	F/38	M/27
Age at onset	30 yr	childhood	childhood	childhood	childhood	25	15	25	12
Exercise int.	+	+	+	+	+	+	+	+	+
Weakness	+	+	-	-	+	+	+	+	+
Myoglobinuria	+	-	-	-	-	-	-	-	+
Family history	-	-	-	-	-	-	-	-	-
Lactic acidosis		+	+	+	+	+	+	+	+
COX+ RRF	+	+	+	+	+	+	+	-	+
cyt b mutation	24del	G14846A	G15168A	G15084A	G15723A	G15615A	G15242A	G15762A	G15059A
a.a. change	8aa del	Gly34Ser	Trpl4lstop	Trpli3stop	Trp326stop	Gly290Asp	Glyl66stop	Gly339Glu	Glyl90stop
% heteroplasmy	50	85	70	87	87	85	63	85	63

Table I. Cliffical features, muscle pathology, and molecular findings in musele mtDNA in 9 patients with exercise intolerance. Only Gomori trichrome stain was used. Patients 1-5 are from Andreu et al, 1999b; patient 6 from Dumoulin et al, 1996; patient 7 from Kennaway et al, 1998; patient 8 from Andreu et al, 1998; and patient 9 from Andreu et al, 1999.

Subunit e	Mutation	Family history	Clinical Manifestations	Reference
COX I	T6742C	negative	acquired sideroblastic anemia	Gattennan et al, 1997
COX I	T6721C	negative	acquired sideroblastic anemia	Gatterman et al, 1997
COX I	G6930A		negative deafness; ataxia; blindness	Bruno et al, 1999
COX I	bpl delta	negative	motor neuron disease	Comi et al, 1999
COX I	G5920A	negative	exercise intolerance, myoglobinuria	Karadimas et al, 1999
COX II	T7587C	maternal	myopathy, ataxia, dementia, optic atrophy	Clark et al, 1999
COX II	T7671A	negative	proximal limb weakness	Rahman et al, 1999
COX III	T9957C	negative	MELAS	Manfredi et al, 1995
COX III	5 bp delta	negative	exercise intolerance, myoglobinuria	Keightley et al, 1996
COX III	G9952A	negative	exercise intolerance, encephalomyopathy	Hanna et al, 1998

Table 2. Clinical features associated with mutations in mtDNA-encoded COX subunits.

Complex	Gene	Mutations	Clinical Picture	Reference
I	*NDUFS4*	5-bp duplication	LS-like	van den Heuvel et al, 1998
I	*NDUFS8*	P79L, R102H	LS (cardiomyopathy)	Loeffen et al, 1998
I	*NDUFV1*	A341V	myoclonic epilepsy	Schuelke et al, 1999
I	*NDUFV1*	R59X, T423M	myoclonic epilepsy	Smeitink & van den Heuvel, 1999
I	*NDUFS7*	V122M	LS	Smeitink & van den Heuvel, 1999
II	flavoprotein	C1684T	LS	Bourgeron et al, 1995
IV	*SURF-1*	multiple	LS	Zhu et al, 1998; Tiranti et al, 1998
IV	SC02	multiple	cardioencephalopathy	Papadopoulou et al, 1999

Table 3. Molecular defects in nuclear genes causing defects in respiratory chain complexes. LS, Leigh syndrome.